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2. Patent application number *(The Patent Office will fill in this part)*	0313892.2	16 JUN 2003
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	Hannah Research Institute Ayr KA6 5HL	
Patents ADP number *(if you know it)*		8653313001
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom	
4. Title of the invention	"Control of Lactation".	
5. Name of your agent *(if you have one)*	Murgitroyd & Company	
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Scotland House 165-169 Scotland Street Glasgow G5 8PL	
Patents ADP number *(if you know it)*	1198013 5	

	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
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Request for substantive examination *(Patents Form 10/77)*	-
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Date 16 June 2003

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"CONTROL OF LACTATION"

The present invention relates to the identification of three peptides which have a regulatory role in the control of milk secretion. The present invention further provides for the use of the identified peptides and antibodies thereto for the control of milk secretion in lactating animals, including humans.

Constituents of milk are known to control the rate of milk secretion according to the frequency and completeness with which those constituents are removed through the demand of the offspring or the farmer's husbandry. This biochemical feedback within the breast or udder acts to modulate the lactation-promoting effects of galactopoietic hormones, and its regulatory characteristics, but not all the active factors in milk, have been described by studies on lactating ruminants at the Hannah Research Institute, Ayr, Scotland.

It has been previously shown that one factor active in goat's and cow's milk is a 7.0-7.6 kDa protein present in a whey protein extract of milk from these animals. This factor was shown to decrease lactose and casein synthesis in cultured explanted pieces of rabbit mammary tissue, and to decrease temporarily the rate of milk secretion when injected into a single mammary gland of the same species via the teat canal.

These studies did not demonstrate a relationship between the concentration of the 7.0-7.6 kDa protein in cow's milk and the animal's rate of milk secretion, and therefore no pivotal role for this protein in the feedback control of milk secretion by milk removal was demonstrated. It has remained a challenge to determine whether there are other inhibitory factors which are present in cow's milk, and which act to match supply of milk with the demand through a process of feedback inhibition.

According to a first aspect of the invention there is provided a peptide including the amino acid sequence RPKHPIKHQG (SEQ ID NO: 1), AVAVSQEAN (SEQ ID NO:2) or SEGVALDPAR (SEQ ID NO:3) or an analogue thereof.

Preferably the peptide is combined with at least one other of the two peptides including the amino acid sequence shown in SEQ ID NO:1, SEQ ID No: 2 or SEQ ID NO:3, this combination reducing milk secretion in animals, including humans.

Preferably the amino acid sequence shown in SEQ ID NO:1, SEQ ID No: 2 and SEQ ID NO:3 is the N-terminal sequence of the peptide.

Preferably the peptide can be co-purified with each of the other peptides including the amino acid sequence shown in SEQ ID NO:1, SEQ ID No: 2 or SEQ ID NO:3 from a 6-30 kDa fraction of whey protein of cow's milk.

In particular, the peptides can be purified from cow's milk by a series of chromatographic separation techniques.

Specifically, when a 6-30 kDa fraction of the whey proteins of cow's milk is resolved by gel filtration on a cross-linked copolymer of allyl dextran and N,N methylenebisacrylamide having an average particle size of 47 µm, such as Sephacryl S-100 (Pharmacia). The fourth-eluting component resolved by this method, "peak S4", comprises the inhibitory peptides.

More specifically, the peptides are co-purified when a nominally 6-30 kDa fraction of the whey proteins of cow's milk is resolved by gel filtration on a cross-linked copolymer of allyl dextran and N,N methylenebisacrylamide having an average particle size of 47 µm, such as Sephacryl S-100 (Pharmacia). The fourth-eluting component resolved by this method, "peak S4", comprises the peptides. When peak S4 is resolved further by peptide gel-

filtration chromatography on a gel of dextran covalently bonded to highly cross-linked agarose beads with a mean diameter of 13-15 µm, such as Superdex Peptide HR (Pharmacia), the leading edge of the major eluted component eluting at 8-11.5 ml, designated P8-11A, contains the inhibitory peptides. Further, when fraction P8-11A is resolved by reversed phase chromatography on a reversed phase column (Genesis 25 cm, C18 4micron; Jones Chromatography), the fractions eluted after 34-36 min at a concentration of 36-39% acetonitrile, in a linear gradient of same in 0.1% trifluoroacetic acid, contains the peptides.

There is further provided a peptide including the amino acid sequence shown in SEQ ID NO:1, SEQ ID No: 2 or SEQ ID NO:3, which in combination with one or more of the other peptides including the amino acid sequence shown SEQ ID NO:1, SEQ ID No: 2 or SEQ ID NO:3 provides a reduction in milk secretion.

In one preferred embodiment, the peptide includes the amino acid sequence shown in SEQ ID NO:1 or an analogue thereof.

In another preferred embodiment, the peptide includes the amino acid sequence shown in SEQ ID NO:2 or an analogue thereof.

In a yet further preferred embodiment, the peptide includes the amino acid sequence shown in SEQ ID NO:3 or an analogue thereof.

A further aspect of the invention provides a peptide mixture comprising two or more different peptides, the peptides including the amino acid sequence shown SEQ ID NO:1, SEQ ID No: 2 or SEQ ID NO:3, or analogues thereof.

Preferably the peptide has a molecular mass determined by mass spectrometric analysis of between 1000 to 3000 Da.

In a particular embodiment of the invention, the peptide is glycosylated.

Alternatively the peptide is unglycosylated.

Further, the peptides of the present invention can be in either phosphorylated or unphosphorylated form.

The present invention further includes truncated versions of the peptides which have been isolated from milk.

Analogues of and for use in the invention as defined herein means a peptide modified by varying the amino acid sequence e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the amino acid sequence may involve insertion, addition, deletion and/or substitution of one or more amino acids, while providing a peptide capable of influencing milk

secretion either on its own, or in combination with other peptides.

Preferably such analogues involve the insertion, addition, deletion and/or substitution of 10 or fewer amino acids, more preferably of 5 or fewer, and most preferably of only 1 or 2 amino acids.

Analogues also include derivatives of the defined peptides, including the peptide being linked to a coupling partner, e. g. an effector molecule, a label, a drug, a toxin and/or a carrier or transport molecule. Techniques for coupling the peptides of the invention to both peptidyl and non-peptidyl coupling partners are well known in the art.

A second aspect of the present invention provides a method of influencing milk secretion in animals, the method including the steps of administering at least one peptide according to the first aspect of the invention.

Preferably the term animal is taken to include humans.

In one preferred embodiment, the animal is a cow, goat or sheep.

A yet further aspect of the present invention provides for antibodies directed to the peptides including the amino acid sequence shown in SEQ ID NO:1, SEQ ID No: 2 or SEQ ID NO:3.

Preferably said antibodies promote or improve lactation in humans and other mammals.

Preferably said antibodies promote or improve lactation in sheep, cows and goats.

An "antibody" is an immunoglobulin, whether natural or partly or wholly synthetically produced. The term also covers any polypeptide, protein or peptide having a binding domain which is, or is homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses and fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies.

The binding member of the invention may be an antibody such as a monoclonal or polyclonal antibody, or a fragment thereof. The constant region of the antibody may be of any class including, but not limited to, human classes IgG, IgA, IgM, IgD and IgE. The antibody may belong to any sub class e.g. IgG1, IgG2, IgG3 and IgG4.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies,

including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that fragments of a whole antibody can perform the function of antigen binding.

Examples of such binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. *et al.*, *Nature* 341:544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.*, *Science* 242:423-426 (1988); Huston *et al.*, *PNAS USA* 85:5879-5883 (1988)); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993)).

The term "antibody" includes antibodies which have been "humanised". Methods for making humanised antibodies are known in the art. Methods are described, for example, in Winter, U.S. Patent No. 5,225,539. A humanised antibody may be a modified antibody having the hypervariable region of a monoclonal antibody such as 791T/36 and the constant region of a human antibody. Thus the binding member may comprise a human constant region.

A yet further aspect of the present invention provides a composition for influencing lactation in animals, the composition including a peptide including the amino acid sequence RPKHPIKHQG (SEQ ID NO: 1), AVAVSQEAN (SEQ ID NO:2) or SEGVALDPAR (SEQ ID NO:3) or an analogue thereof.

Preferably the animal is a non-human animal.

More preferably the animal is a cow, goat or sheep.

Preferably the composition inhibits lactation in the target cells within hours of administration, with the response dependent on the frequency of milk removal from the mammary gland.

Preferably the composition is administered by intra-ductal injection into the mammary gland at a dose level yielding a final concentration of peptides in milk in the range 0.1 to 10 micromolar. The administration of this dose may be repeated as

required, and possibly increased when given over long periods.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*.

In summary, the present invention provides the surprising and unexpected finding that at least one peptide which can act in combination with other defined peptides, inhibits the secretion of milk constituents in primary cell cultures that reproduce the activities of lactating mammary tissue.

Description of the drawings

The present invention will now be described, by way of example only, with reference to the accompanying drawings, wherein;

Figure 1 shows the resolution of the 6-30 kDa whey fraction by Sephacryl gel filtration chromatography,

Figure 2 shows the further resolution of gel-filtration peak S4 by Superdex peptide chromatography,

Figure 3 shows an example of the further resolution of the Superdex fraction P8-11A by reversed phase HPLC,

Figure 4 shows the inhibition of protein secretion in acini cultures by fractions obtained through resolution of a 6-30 kDa fraction of cow's whey proteins by Sephacryl gel filtration chromatography,

Figure 5 shows the inhibition of protein secretion in cell culture by fractions of prepared by Superdex high-resolution peptide chromatography,

Figure 6 shows inhibition of protein secretion in mammary acini cultures by components of peptide fraction 8-11A resolved by reversed phase HPLC,

Figure 7 shows the effect of peptide A on protein secretion in mammary cell cultures,

Figure 8 shows the effect of peptide B on protein secretion in mammary cell cultures,

Figure 9 shows the effect of peptide C on protein secretion in mammary cell cultures, and

Figure 10 shows the effect of a combination of peptides A, B and C on protein secretion in mammary cell cultures.

Detailed Description

The peptides of the invention exist in cow's milk, possibly in glycosylated or phosphorylated form.

The peptides may act together to inhibit the rate of milk secretion in the mammary gland.

The peptides of the invention can be obtained from cow's milk by a method described herein or by some variant thereon. It has been demonstrated that the three peptides of the invention isolated from cow's milk are able to inhibit the secretion of milk proteins in mammary acini cultures. When the three peptides are present together in a milk fraction added to the culture medium for a two hour period, they are able to inhibit protein secretion in a concentration-dependent manner.

Synthetic peptides based on the N-terminal sequence of the natural peptides can be synthesised by standard Fmoc amino acid chemistry. Synthetic peptides produced according to the N-terminal sequence of the peptides of the invention, and representing truncated 9- or 10-amino acid forms of the natural peptides similarly inhibit the secretion of protein in primary cultures of mammary cells prepared as acini by collagenase digestion of lactating tissue. Inhibition is exerted acutely, within two hours, and is elicited in a concentration-dependent manner by synthetic peptides in combination. It is expected that the inhibitory activity of synthetic peptides will depend on the proportion of the full-length sequence synthesised and that the inhibitory potency of these and the natural peptides will depend on the degree of

peptide modification by glycosylation or phosphorylation.

The invention is applicable to any animal responsive to the inhibitory peptides defined herein. In addition, the demand-led relationship between milk supply and milk removal in most if not all mammals predicts that the same effects will be demonstrable in relation to the peptides of the invention obtained from milk of other species, in relation to that species. In man, administration by a suitable route of the peptides, or antibodies thereto, may be applied to improve or suppress lactation. In dairy cows, there maybe a need to reduce milk yield in order to maintain production within quota limits, in which case the inhibitory peptides themselves are administered. For intra-ductal injection of peptides into the mammary gland, a dose yielding a final concentration of peptides in milk in the range 0.01-1.0 micromolar is likely to be effective, and should be repeated as required, and possibly increased when given over long periods.

Conversely, passive immunisation methods using antibodies against the peptides may be used to generate a reduction in the effect of the natural inhibitory peptides when this is desired in order to increase milk supply in lactating animals.

Antibodies against the natural peptides of the invention or against their synthetic analogues can be raised by conventional methods e.g. as polyclonal

antisera, mouse monoclonal antibodies, cow-mouse hybrid monoclonal antibodies or as engineered antibodies, by any of the currently available methods. Conventional carriers and adjuvants known in vaccination can be used. Antibodies against synthetic truncated peptides based on the sequence of the peptides of the invention may be used to isolate the natural peptides from cow's milk extracts, or to control milk supply as described above.

EXAMPLE

Preparation of cow milk fractions

Milk was collected at the morning milking from Friesian cows, and was defatted by centrifugation (2500g, 15°C, 20 min) and filtered through glass wool. Casein in defatted milk was precipitated by dropwise addition of concentrated HCl until the pH reached 4.6. After stirring for 10 min, casein was sedimented by centrifugation (2500g, 15 °C, 20 min), and the clear whey supernatant was filtered through glass fibre membranes of decreasing pore size, the final membrane made of polyethersulphone having a cut-off of 0.45 microns. The whey fraction was subjected to ultrafiltration using a filter with a nominal cut-off value of molecular weight30,000 Daltons (Da). The filtrate was dialysed for 24 h against 10 mM sodium acetate buffer pH 4.6 containing 1.5mM ε-aminocaproic acid, 100 μM glutathione, 1mM EDTA and 1mM EGTA using a dialysis membrane with a nominal molecular weight cut-off of

6,000 Da, and was then adjusted to pH 7.0 by addition of NaOH. The neutralised filtrate was dialysed against 2mM phosphate buffer pH 7.0 containing 1.5mM ε-aminocaproic acid, 100 μM glutathione, 1mM EDTA and 1 mM EGTA for 24 h and then freeze dried.

Gel Filtration Chromatography

The 6-30kDa whey fraction was resolved of a Hi-Prep Sephacryl S-100 High-Resolution gel filtration column (Pharmacia) using a Fast Protein Liquid Chromatography (FPLC) system (Pharmacia). The freeze-dried whey fraction was reconstituted in one tenth its volume before freeze-drying, and the solution was clarified by filtration through a 0.22 μm filter. The chromatography buffer was 20 mM phosphate buffer pH 7.0 containing 0.15 m NaCI, and was filtered through a 0.22 μm filter and degassed before use. Two ml of the 10 x concentrated whey fraction was loaded for each separation. The flow was 1ml/min.

Fractions containing protein peaks eluted from the column were tested for inhibitory activity in a cell culture bioassay (see below), and fractions spanning one protein peak containing inhibitory activity, designated peak S4, were combined and desalted by passage through a column composed of Poros 50 R2, composed of cross-linked poly(styrene-divinylbenzene (PerSeptive Biosystems). Protein bound to the column was washed with distilled water, and then

eluted with 80% (v/v) acetonitrile in distilled water. The inhibitory fraction was then concentrated under a stream of nitrogen and freeze dried. A second peak of inhibitory material, designated peak S6, contained too little protein for further purification to be practicable.

Fraction S4 was resolved further on a Superdex Peptide HR 10/30 column (Pharmacia) using an FPLC system. Dried fraction was reconstituted in 0.2-0.4 ml of solution of 0.2 mM AEBSF, 10 mM EDTA and 10 mM EGTA, and chromatography was performed in 20 mM phosphate buffer pH 7.0 containing 0.25 M NaCl at a flow rate of 1 ml/min. Fractions containing protein peaks eluting from the column were collected. These were either desalted on a Poros column and freeze dried as described above for assay of inhibitory activity, or were freeze dried immediately for further fractionation. Inhibitory material was detected in the leading edge of the major peak, designated fraction P8-11A.

Fraction P8-11A was resolved further using a reversed phase HPLC column Genesis 25 cm C18, 4 μ; Jones chromatography) on a Spectra Physics HPLC system. Sample containing the equivalent of one Superdex fraction P8-11A was dissolved in water and loaded in a volume of 0.2 ml. The column was eluted with a gradient of 0-60% acetonitrile in 0.1% (w/v) trifluoroacetic acid (TFA), and 1 ml fractions of the eluate were collected. HPLC fractions were

freeze dried, and tested for inhibitory activity as described above.

Mammary cell culture bioassay of milk fractions

Mammary cells were prepared from tissue of lactating mice by collagenase digestion according to the method of K Hendry, K Simpson, K Nicholas & C Wilde. Journal of Molecular Endocrinology 21: 169-177 (1998). The resultant suspension of mammary acini consisted predominantly of groups of 50-200 cells, and was cultured in medium (Medium 199/Ham's F12: 50:50 v/v) containing insulin (5 μg/ml), hydrocortisone (0.1 μg/ml) and prolactin (1 μg/ml). Culture density was 1.5 x 10^6 cells/ml, and cells were maintained at 37°C in an atmosphere of 5% CO_2 in air. Protein synthesis and secretion were measured by continuous labelling with L-[4.5-3H]leucine (40-70 mCi/mmol; 10-20 μCi/ml) for 2 h in the presence or absence of milk fractions at concentrations of 0.2 - 4.0 μg/ml) or synthetic peptides (0.01 - 10 μM). Milk extracts or synthetic peptide were dissolved and diluted in 10 mM Hepes buffer pH 7.4, and control cultures containing only diluent were included in each experiment. The culture was terminated by centrifugation of the cell suspension (2000g, 2 min), and the cell pellet and supernatant were frozen and stored separately for assay of DNA and protein secretion respectively. Radiolabel incorporation was measured by precipitation with trichloroacetic acid (final concentration 10% w/v). Cell lysates were prepared by sonication using a

Kontes KT50 cell disrupter (setting 20, 15 s) in 0.1 M NaH_2PO_4 pH 7.4 containing 2 M NaCl, and assayed for DNA content by the method of C Labarca and K Paigen, Analytical Biochemistry 102: 344-352 (1980). Secretory activity was expressed per unit of cellular DNA.

The amount of radiolabelled protein secreted by acini in the presence of milk extracts or synthetic peptides was expressed as a percentage of that produced by the cells to which no milk fraction or peptide was added. In each experiment, treatments were replicated in a minimum of three culture wells, and results for individual experiments were mean values for those wells. Values shown in Figures 4-6 are the mean for three or four experiments, each testing a different preparation of milk fractions. Error bars show the standard error of the mean for these experiments.

Figure 4 shows the inhibition of protein secretion in acini cultures by fractions obtained through resolution of a 6-30 kDa fraction of cow's whey proteins by Sephacryl gel filtration chromatography. Figure 5 shown the inhibition of protein secretion in cell culture by fractions of prepared by Superdex high-resolution peptide chromatography.
Figure 6 shown inhibition of protein secretion in mammary acini cultures by components of peptide fraction 8-11A resolved by reversed phase HPLC.

Preparation of synthetic peptides based on natural peptide sequences

N-terminal sequencing of the inhibitory HPLC fraction eluting at 36-39% acetonitrile revealed three peptides with the N-terminal sequences:

Peptide A: RPKHPIKHQG
Peptide B: AVASQEAN
Peptide C: SEGVALDPAR

Peptide A was identified as the N-terminal sequence of α_{s1}-casein. Peptides B and C have limited homology to known amino acid sequence data (SwissProt protein sequence database). Mass spectrometric analysis indicated that peptides had masses in the range 1000 - 3000 Daltons.

Peptide A was produced synthetically by chymosin digestion of α_{s1}-casein purified by a modification of the method of E. Lahov and W Regelson, Food Chemistry and Toxicology 34:1 131-145 1996. The N-terminal fragment of mass 2762 was isolated from the chymosin digest by reversed phase chromatography system. This peptide consists of a 23 amino acid sequence at the N-terminus of the α_{s1}-casein protein.

N-terminal sequences of Peptides B and C comprising nine and ten amino acids respectively were synthesised by solid phase synthesis using Fmoc amino acids coupled by the PyBOP/HOBt/DIPEA method. The peptides were cleaved from the resin with 80%

TFA plus suitable scavengers and purified by reverse phase HPLC on a Phenomenex Luna 10 µ C18 column of dimensions 25cm x 0.212cm using a linear gradient of water to 100% acetonitrile in 0.1% TFA.

Mammary culture bioassay of synthetic peptides

Protein secretion was measured in mammary acini cultures in the presence and absence of synthetic peptides. Peptides were added singly or in combination at equimolar concentrations over a range of 0.01 - 10.0 µM. In each experiment, treatments were replicated in a minimum of three culture wells, and results for individual experiments were mean values for those wells. Values shown in Figures 7-9 are the mean for three experiments. Error bars show the standard error of the mean for these experiments.

Statistical analysis of bioassay data showed that, together, the three peptides exhibited a concentration-dependent inhibition of secretion. Maximal inhibition of secretion was obtained at a concentration of 0.1-1.0µM of each peptide. None of the peptides tested individually inhibited secretion to this extent, and at higher concentrations the inhibitory action of peptide A was counteracted by a stimulatory effect of peptide B. The effects of the synthetic peptides indicate that inhibition of secretion by the HPLC fraction containing natural peptides with the same N-terminal sequences is due to the combined actions of the peptides, and is

unlikely to be conferred by any one constituent of the active HPLC fraction.

Figures 7, 8 and 9 show the effect of peptides A, B ~~and C on protein secretion in mammary cell cultures.~~ Figure 10 shows the effect of a combination of peptides A, B and C on protein secretion in mammary cell cultures.

All documents referred to in this specification are herein incorporated by reference. Various modifications and variations to the described embodiments of the inventions will be apparent to those skilled in the art without departing from the scope of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes of carrying out the invention which are obvious to those skilled in the art are intended to be covered by the present invention.

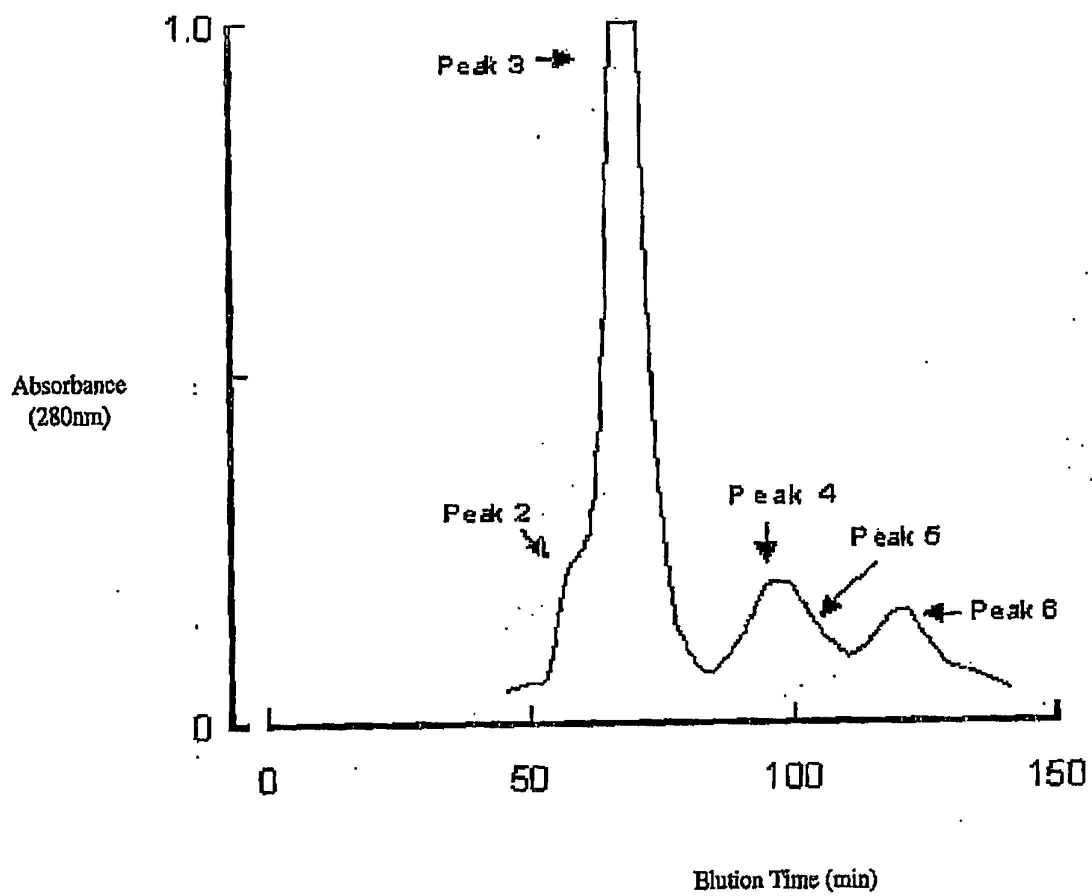

Figure 1.
Gel filtration chromatography of bovine 6-30 kDa whey fraction

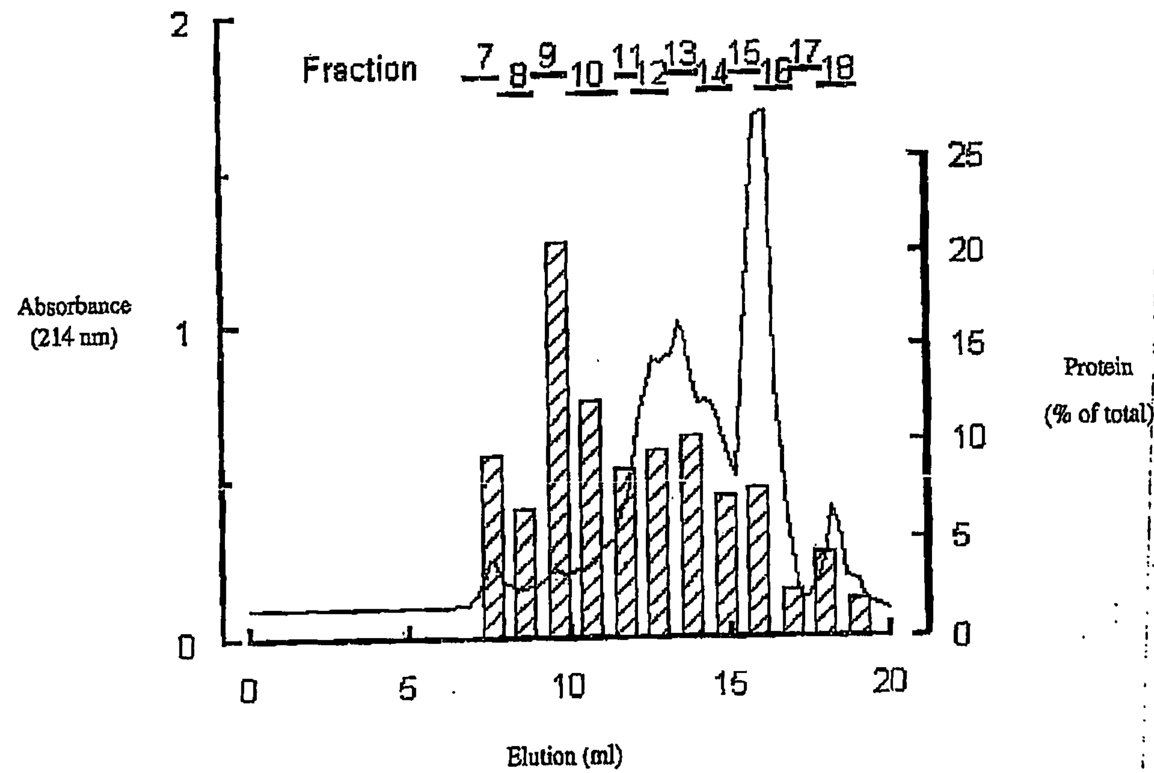

Figure 2. Resolution of inhibitory peak S4 by peptide chromatography

3/10

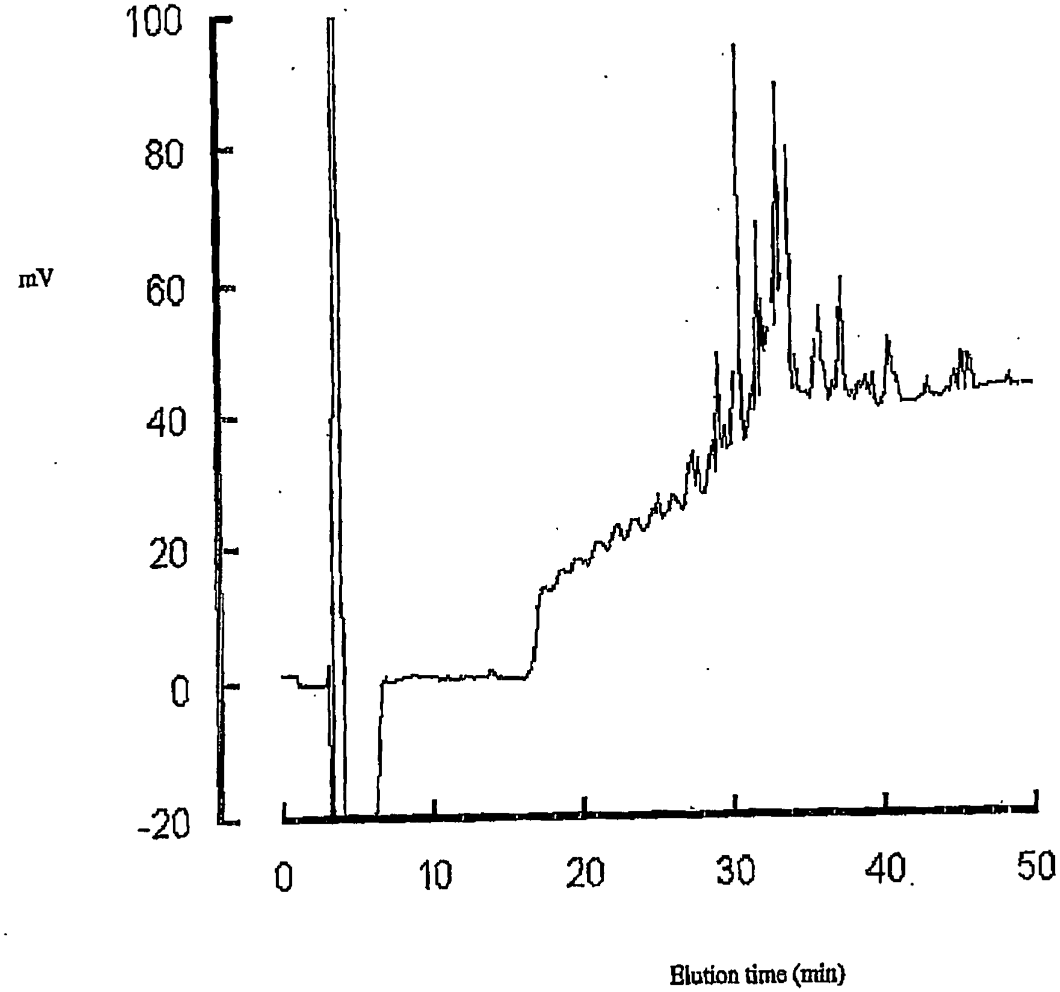

Figure 3. Resolution of peptide fraction P8-11A by reversed phase chromatography

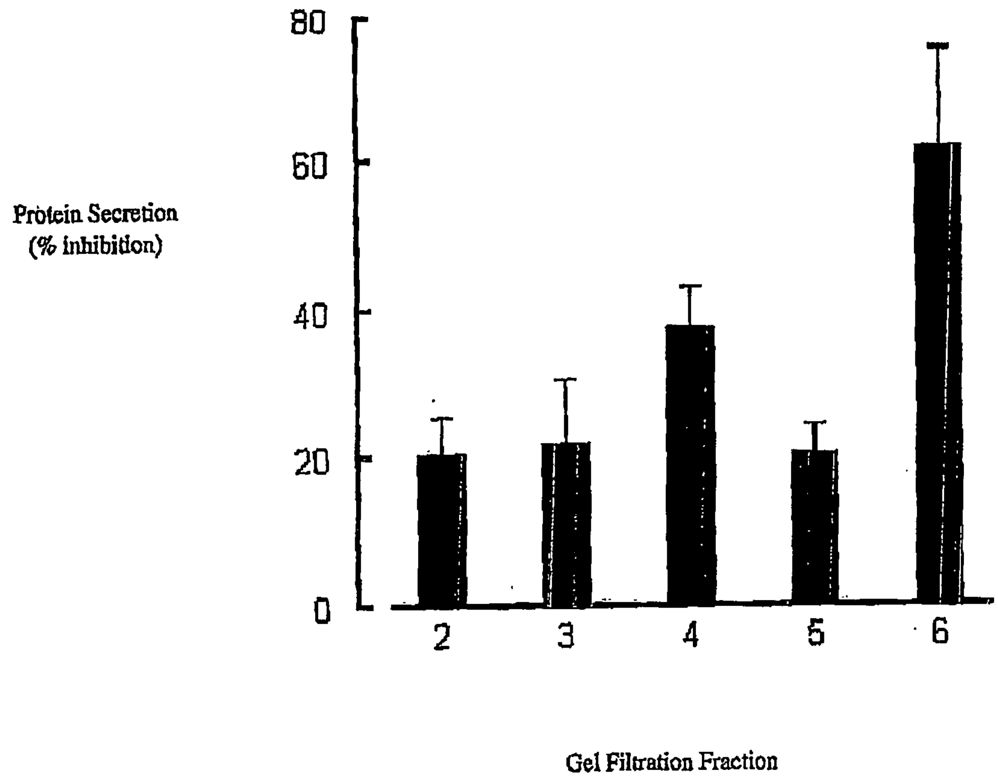

Figure 4. Bioassay of gel-filtered whey fractions

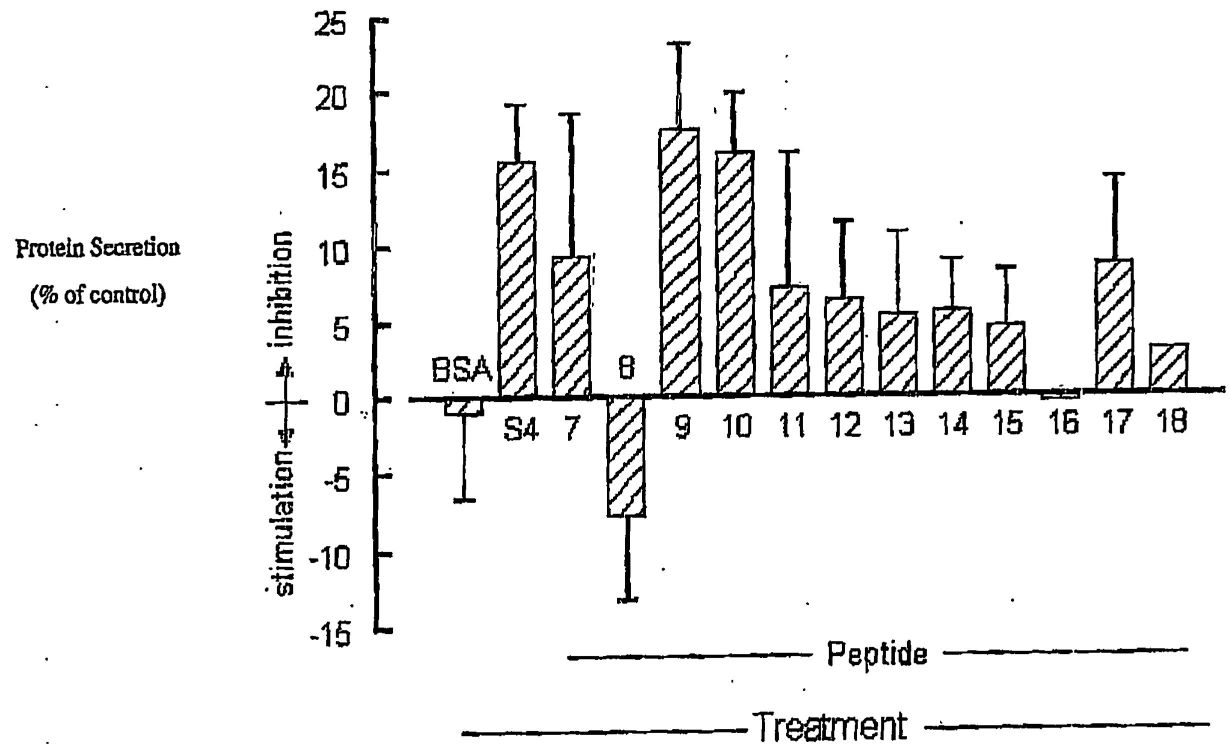

Figure 5. Inhibition of protein secretion by bovine whey peptide fractions

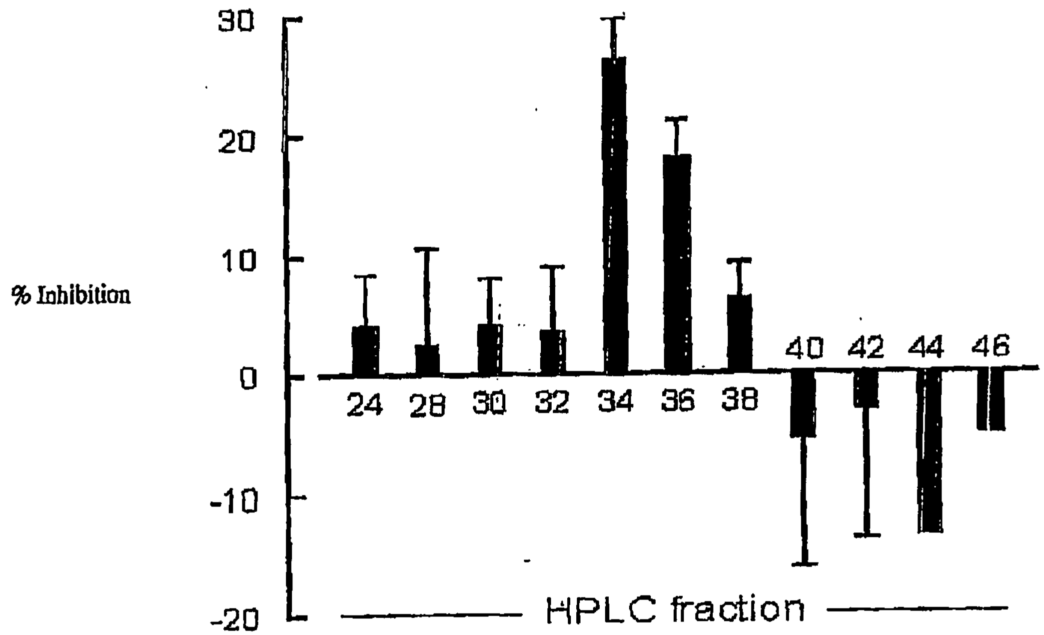

Figure 6. Inhibition of protein secretion by HPLC-resolved fractions

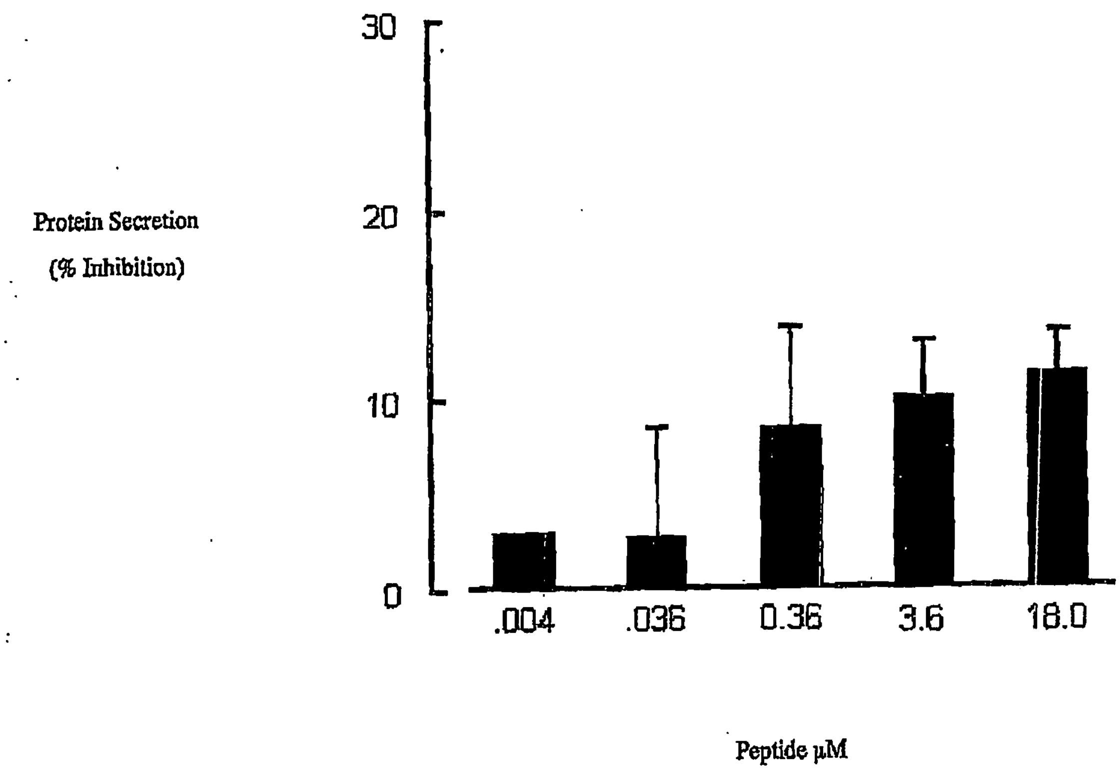

Figure 7. Inhibition of protein secretion in mammary cell cultures by synthetic peptide A

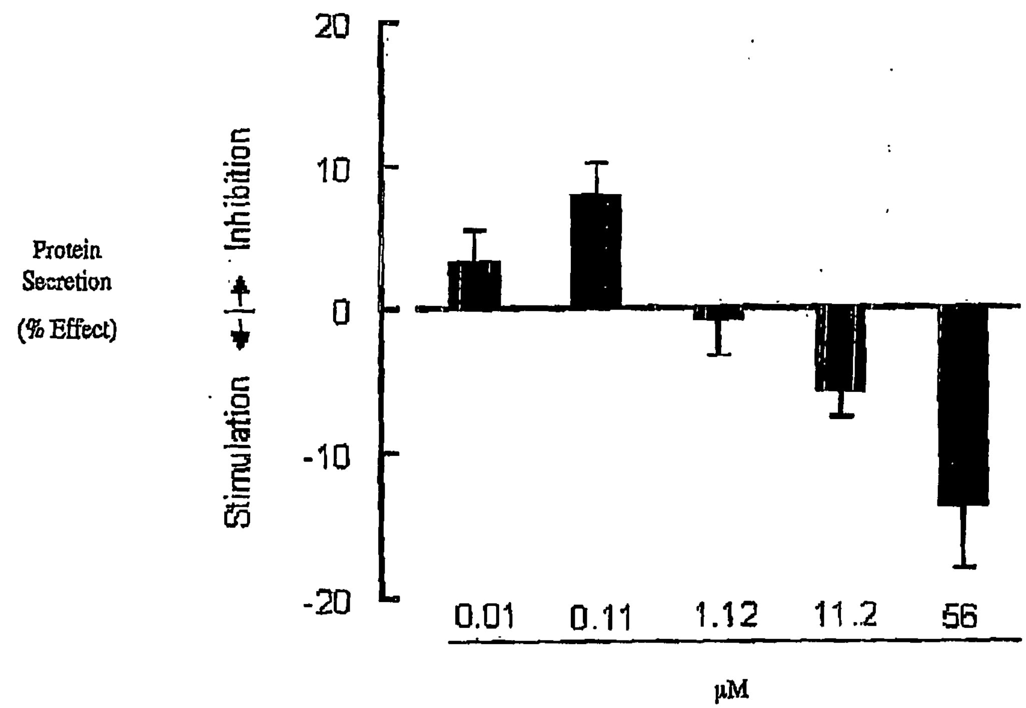

Figure 8. Inhibition of protein secretion in mammary cell cultures by synthetic peptide B

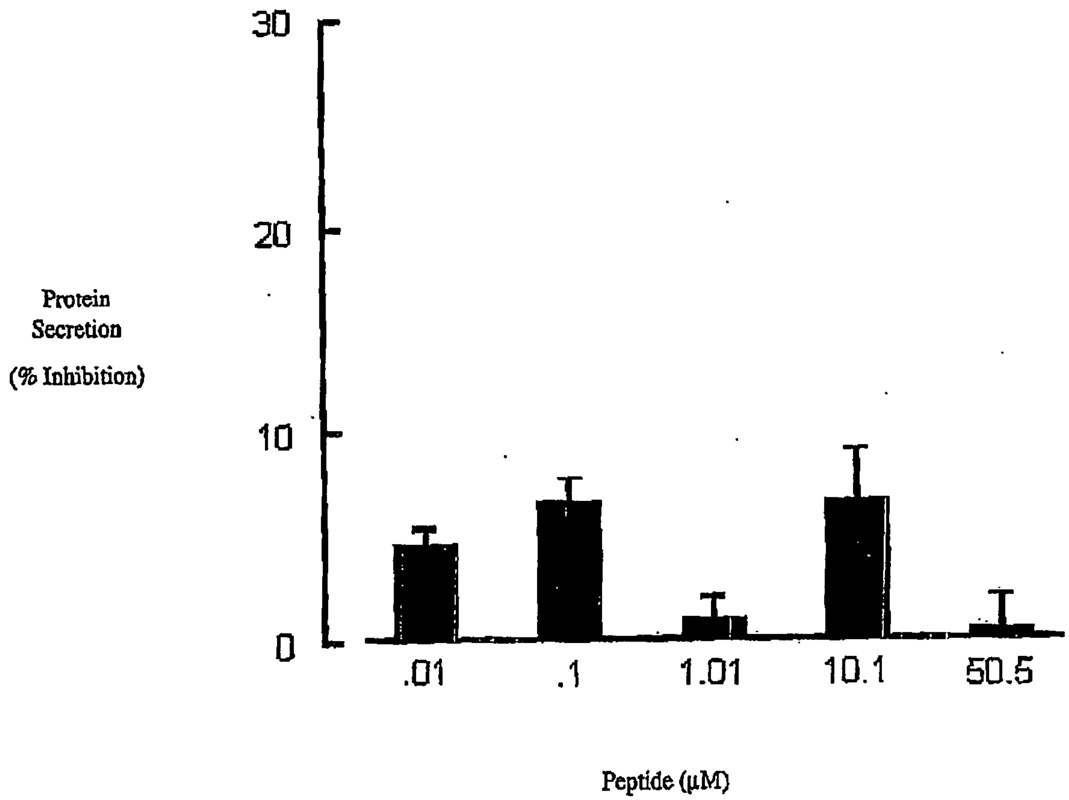

Figure 9. Inhibition of protein secretion in mammary cell cultures by peptide C

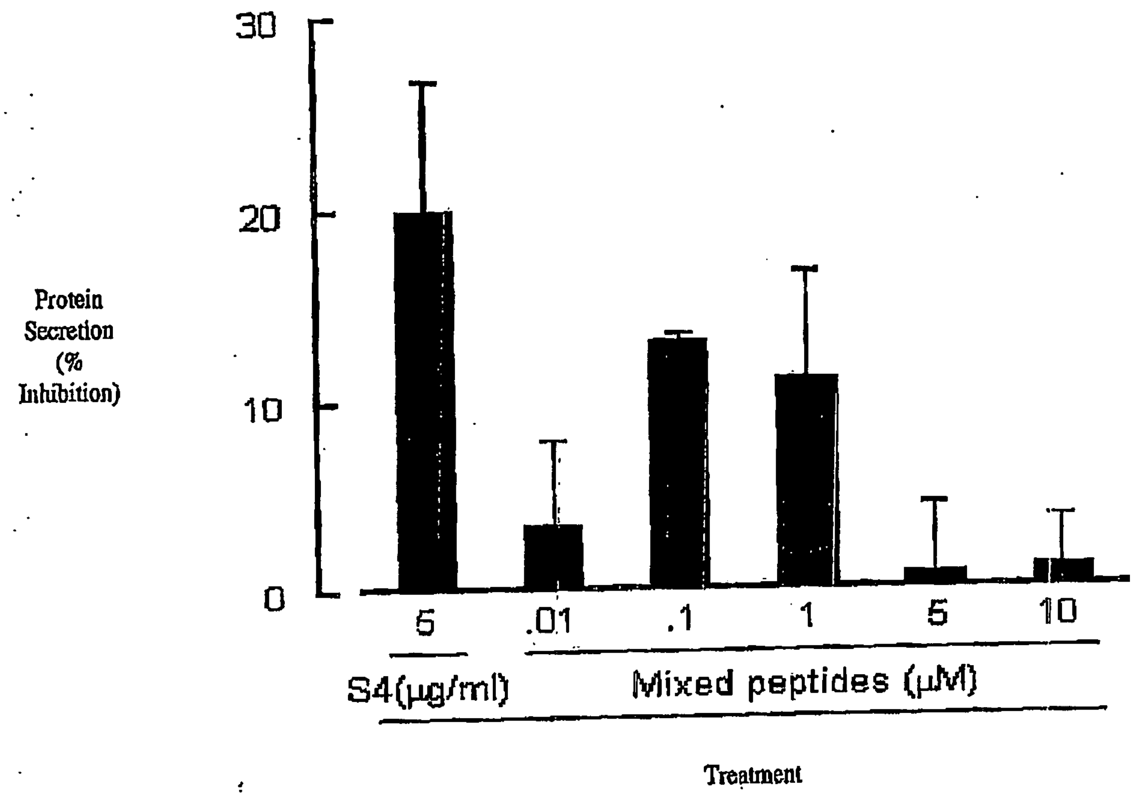

Figure 10. Inhibition of protein secretion in mammary cell cultures by a combination of synthetic peptides A, B and C.